NCI ETI Branch
Flow Cytometry Core Laboratory

Immunolabeling of isolated nuclei for flow cytometric analysis.

A number of laboratories have described nuclei isolation protocols for DNA content analysis, eliminating the need for DNA dyes to cross a permeablized plasma membrane reducing non-specific dye binding to cytoplasmic components, allowing very accurate DNA cell cycle analysis. Isolation of stable nuclei that can be analyzed on a flow cytometer is also a useful technique for studying proteins that translocate to the nucleus, including nuclear transcription factors. We have been experimenting with techniques for isolating stable cell nuclei from mammalian cells, allowing them to be labeled for intranuclear antigens and analyzed on the flow cytometer. Using these techniques, we have been able to measure heat shocked-induced translocation of the chaperonin hsp70 to the nucleus as a function of total cellular chaperonin content. These techniques utilize the multilaser capabilities of the FACSVantage, using our HeNe laser and red-excited probes for best sensitivity. Techniques for detecting NFkB component translocation have also been developed and are currently under review.

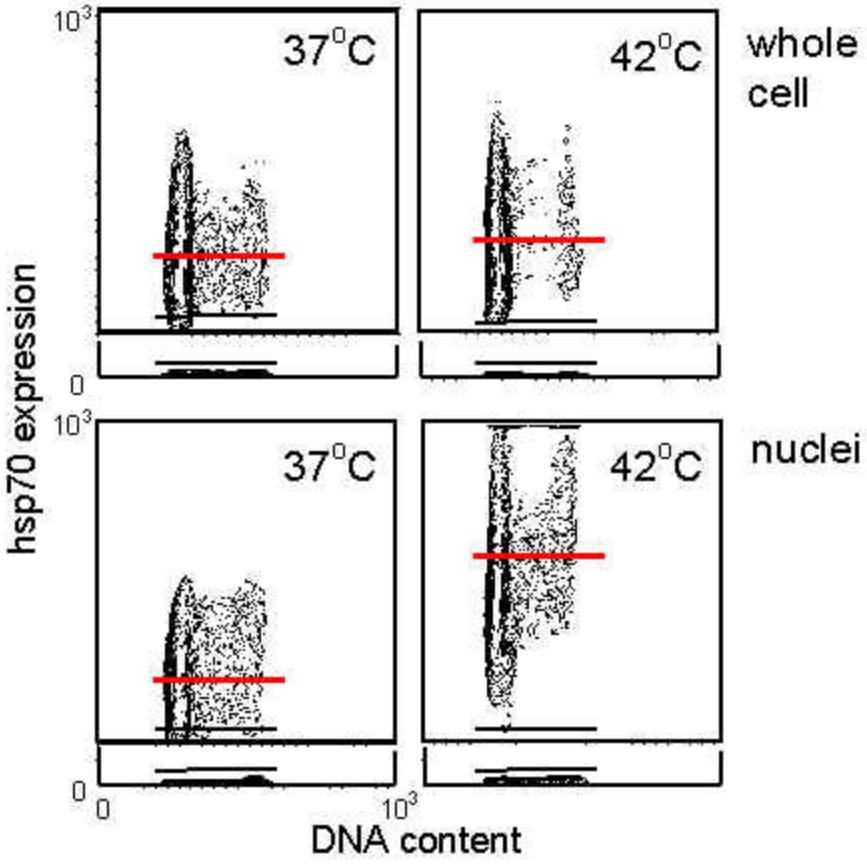
Protocol

1. The cell type of interest (in this case, mouse L929 cells) are incubated for 1 hour either at 37C or 42C. The cells are then removed from their dishes by gentle scraping and washed twice in cold PBS.
2. Cells are then resuspended in cold nuclei extraction buffer (320 mM sucrose, 5 mM $MgCl_2$, 10 mM HEPES, 1% Triton X-100 at pH 7.4) at approximately 1 ml per 1 million cells. The cells are gently vortexed for 10 seconds and allowed to incubate on ice for 10 minutes. No dounce homogenization is necessary. Nuclei were then pelleted by centrifugation at 2000 x g and washed twice with nuclei wash buffer (320 mM sucrose, 5 mM $MgCl_2$, 10 mM HEPES at pH 7.4, *no Triton X-100*). Nuclei yield and integrity were confirmed by microscopic examination with trypan blue staining. For all cell types tested (mainly connective and lymphoid tissues), this extraction procedure gives greater than 98% successful lysis with little debris and minimal clumping.
3. The resulting nuclei can then be subdivided and immunolabeled for the antigen of interest. For the data below, nuclei were incubated overnight with an anti-hsp70 monoclonal antibody followed by two hours incubation with Cy5-anti-mouse IgG. The labeling buffer should be the nuclei wash buffer described above with 1% BSA and 0.1% sodium azide added. Magnesium chloride maintains nuclei integrity and should not be omitted from any buffers used to label nuclei. Nuclear integrity can be maintained for at least 24 hours using the above buffer at 4C.

4. The cells are then counterstained with propidium iodide (PI) at 50 ug/ml with RNase at 100 U/ml to measure DNA content and analyzed on a multibeam flow cytometer (such as a FACSVantage or FACSCalibur) exciting the PI with an argon-ion laser at 488 nm and Cy5 with a red diode or helium-neon laser emiting at 632 nm.
5. For comparison of "total" cellular versus nuclear antigen content, intact EtOH-fixed cells can also be labeled for hsp70 expression and analzyed alongside the nuclear preparation (as describe below).

Results

(Below). Detection of hsp70 expression and nuclear translocation in untreated and heat-shocked L929 cells by flow cytometry. Untreated or heat-shocked mouse L929 cells were either fixed intact with 70% EtOH, or membrane-lysed to extract intact nuclei as described above. Both whole cells and nuclei were immunolabeled with anti-hsp70 antibodies, followed by secondary antibody labeling with Cy5-anti-mouse IgG and counterstaining with propidium iodide. The cells were then analyzed on a FACSVantage using primary argon-ion 488 nm and secondary helium-neon 633 nm secondary laser beams. Although heat treatment caused little increase in total cellular hsp70 content (top panels), considerable nuclear localization of existing cytoplasmic pools occured at 42C (bottom panels).



Flow cytometric antibodies against components of the NFkB nuclear transactivating complex have been identified and are currently being accessed for their ability to detect NFkB translocation.

This protocol was prepared by the Telford Lab for the NCI ETI Branch and its friends. 1-27-00